CUTICULAR HYDROCARBONS OF *Glossina*, III: SUBGENERA *Glossina* AND *Nemorhina*

BRUCE D. SUTTON[1] and DAVID A. CARLSON[2, *]

[1]*Division of Plant Industry, Florida Department of Agriculture & Consumer Services*
Gainesville, Florida 32614

[2]*USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology*
Gainesville, Florida 32604

Abstract—The cuticular methyl-branched alkanes of tsetse flies of the subgenera *Glossina* (sensu stricto, formerly *morsitans*) and *Nemorhina* (formerly *palpalis*) were identified and quantified by capillary gas–liquid chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Males of *Glossina* (*Nemorhina*) are differentiated from *G.* (*Glossina*) by dominant 27-, 28-, and/or 29-carbon backbone trimethylalkanes with the methyl positions at 3,7,11-, 4,8,12-, and 3,7,11-, respectively. All females contain major quantities of long-chain internally branched di- and/or trimethylalkanes that were previously implicated as mediators of sexual behavior in males. Taxa within these two subgroups that are closely related and/or conspecific, based upon conventional morphological and ecological criteria, exhibit similar GC patterns and similar internally branched di- and trimethylalkane isomers in females. Examination of these potentially stimulatory methylalkanes may provide reasons for the reproductive isolation of closely related species from each other.

Key Words—Tsetse fly, sex pheromone, gas–liquid chromatography, mass spectrometry, methylalkanes, patterns, Diptera, hydrocarbons.

INTRODUCTION

The cuticular lipids of the tsetse fly (*Glossina* Wiedemann) (Diptera: Glossinidae) have come under scrutiny as a result of the identification of hydrocarbon constituents having biological activity. The existence of a female-produced compound in the epicuticular waxes of the tsetse fly capable of stimulating male sexual behavior was first demonstrated for *Glossina morsitans morsitans* West-

*To whom correspondence should be addressed.

wood (Langley et al., 1975). The compound found to be most active in initiating male copulatory activity in the lab was isolated and identified as a trimethylalkane, 15,19,23-trimethylheptatriacontane (Carlson et al., 1978). Lesser activity was reported for two dimethyl-branched compounds, 15,19- and 17,21-dimethylheptatriacontane.

A sex stimulant pheromone was also demonstrated in adult female *G. pallidipes* Austen (McDowell et al., 1981, Langley et al., 1982), and the compound eliciting the maximal response in males of this species was identified as 13,23-dimethylpentatriacontane (Carlson et al., 1984). Males of *G. austeni* Newstead were reported to exhibit a copulatory response to synthetic 15,19-dimethyltritriacontane (Huyton et al., 1980), although the identity of a sex pheromone in this species remains ambiguous. In addition, epicuticular constituents have been suggested as promoting sexual activity in *G. palpalis palpalis* Robineau-Desvoidy (Offor et al., 1981). Two unusual dimethylalkanes, 11,23- and 13,25-dimethylheptatriacontane, that have 11 methylene units between the methyl branches were found in *G. tachinoides* Westwood females as an inseparable mixture (Nelson et al., 1988). The natural hydrocarbons extracted from female *G. tachinoides* were stimulatory to males (Messoussi et al., 1994), but only recently have these compounds been synthesized (Matsuyama and Mori, 1994) and available for bioassay. Initial results (Carlson, unpublished data) indicate that synthetic 11,23- and 13,25-dimethylheptatriacontane release sexual responses in male *G. tachinoides*.

The identification of long-chain methylalkanes in the hydrocarbon constituent of epicuticular waxes in tsetse flies that exhibited highly specific behavioral activity and the likelihood that such compounds would be species specific stimulated the initial study of the cuticular hydrocarbon patterns and composition of a number of *Glossina* taxa. Species of the subgenus *Glossina* Wiedemann sensu stricto (the *morsitans* group; *G. m. morsitans*, *G. austeni* and *G. pallidipes*) have been reported to have cuticular methylalkane compositions comprised of homologous series of externally branched monomethylalkanes (2- and 3-methylalkanes), internally branched monomethylalkanes, dimethylalkanes, and trimethylalkanes (Nelson and Carlson, 1986). There were only low levels of *n*-alkanes detected. The 2-methylalkanes were predominant in males, while the females were found to possess, in addition to the 2-methylalkanes, substantial quantities of internally branched dimethyl- or trimethylalkanes corresponding to the putative sex pheromones.

Glossina fuscipes fuscipes Newstead, *G. palpalis palpalis*, *G. p. gambiensis* Vanderplank, and *G. tachinoides*, of the subgenus *Nemorhina* Robineau-Desvoidy (the *palpalis* group), have also been investigated (Nelson et al., 1988). The females of these taxa were similar to those of *G. (Glossina)* in that the patterns were dominated by homologous series of 2-methylalkanes together with series of internally branched di- or trimethylalkanes. Males were characterized

by patterns having substantial amounts of 27- or 29-carbon backbone 3,7,11-trimethylalkanes and a 28-carbon backbone, 4,8,12-trimethylalkane in addition to the 2-methylalkane series. The single species of the subgenus *Austenina* Townsend (the *fusca* group), *G. brevipalpis* Newstead, for which the cuticular methylalkanes have been determined, exhibits a pattern quite distinct from the species of *G.* (*Glossina*) and *G.* (*Nemorhina*) (Nelson et al., 1988). In *G. brevipalpalis*, tetramethylalkanes are a significant component of the methylalkanes, with 3,7,11,15-tetramethylhentriacontane the predominant isomer, and the internally branched compounds in minor quantities. In recent trials of the hydrocarbon fraction from females of this species, conspecific colony males were strongly sexually stimulated, implicating 3,7,11,15-tetramethylalkane homologs in modifying sexual behavior in this species (D.A.C., unpublished data).

It is possible to elucidate the possible taxonomic correlations in methylalkane patterns within the genus *Glossina* and to identify female-specific methylalkanes having potential for biological activity in taxa not yet investigated. Examination of these potentially stimulatory methylalkanes may provide reasons for the reproductive isolation of closely related species from each other, by the presence or absence of essential components. Thus we have analyzed the hydrocarbons of all but one of the remaining taxa of the *Glossina* and *Nemorhina* subgenera.

METHODS AND MATERIALS

The methylalkane composition was determined for adult males and females of the following species: *G. morsitans centralis* Machado, *G. m. submorsitans* Newstead sensu Machado, *G. longipalpis* Wiedemann and *G. swynnertoni* Austen of *G.* (*Glossina*) s. str. together with *G. caliginea* Austen, *G. fuscipes martinii* Zumpt, *G. pallicera pallicera* Bigot, and *G. p. newsteadi* Austen of *G.* (*Nemorhina*). Specimens of the sole remaining member of *G.* (*Nemorhina*), *G. fuscipes quanzensis* Pires, were not available for analysis.

Each dried specimen was extracted in hexane and the hydrocarbons isolated from other lipid components by elution with hexane (3 ml) using silica gel chromatographic columns packed in Pasteur pipets (50 mm × 6 mm ID) as previously described (Carlson et al., 1984). The alkanes were obtained by elution with hexane (3 ml) from 5% silver nitrate-impregnated silica gel columns (50 mm × 6 mm ID). Analysis of cuticular components involved quantification of eluents by gas-liquid chromatography (GC) utilizing a 50-m × 0.15-mm-ID fused-silica capillary column having a 0.25-μm dimethyl siloxane stationary phase (BP-1, Scientific Glass Engineering Inc., Austin, Texas) fitted to a Tracor 540 gas chromatograph (Tracor, Austin, Texas) with a cool on-column injector

(OCI-3, Scientific Glass Engineering) and flame-ionization detector. H_2 carrier was used with a linear flow velocity of 35 to 45 cm/sec. Each sample was reconstituted in 10–70 μl of hexane as necessary to provide optimal and consistent stationary phase loading, and 1–2 μl was injected. Each GC run was temperature programmed as follows: initial hold at 60°C (2 min), ramp 20°/min to 230°C, then 1°/min to 350°C, final hold (20 min). Electron ionization (EI) and chemical ionization (CI) mass spectra were obtained using Hewlett-packard 5988A MS interfaced to a HP 5890 gas chromatograph (GC) fitted with an OCI-3 injector. The column utilized was 30 m × 0.32 mm ID (DB-1, J&W Scientific, Folsom, California) having a 0.25-μm polymethylsiloxane stationary phase. He carrier was used at a linear velocity of 40 cm/sec. The MS interface was maintained at 315°C, electron voltage at 70 eV, and the system parameters manually optimized in order to enhance the high-mass spectra. The mass spectral scan range extended from m/z 70 to m/z 750 at 1 scan/sec. MS scans were manually background subtracted prior to interpretation. Methane (700 torr) was used as the reagent gas for CI (electron voltage at 30 eV, ion source temperature at 100°C).

The identification of cuticular methylalkane components based upon EI mass spectra followed the conventions for their interpretation, including the following for types and partial locations of branches; A = internal monomethyl, B = internal dimethyl, C = internal trimethyl, D = internal tetramethyl, A' = 2- (3- or 4-) methyl, B' = 3,7-dimethyl, and C' = 3,7,11- or 4,8,12-trimethyl. (Carlson et al., 1984; Nelson and Sukkestad, 1970; Nelson and Carlson, 1986; Nelson et al., 1980, 1988). The CI mass spectra were used to assign molecular weights to eluents.

Label data for all specimens are given in Appendix 1. Specimens are vouchered at the The Natural History Museum, London, U.K. [NHM, formerly British Museum (Natural History), or the Florida State Collection of Arthropods, FDACS, Gainesville, Florida, (FSCA)].

RESULTS

The n-alkanes and methylalkanes identified by GC-MS from *G. morsitans centralis*, *G. m. submorsitans*, *G. longipalpis*, and *G. swynnertoni* of the subgenus *Glossina* and *G. caliginea*, *G. fuscipes martinii*, *G. pallicera pallicera*, and *G. p. newsteadi* of the subgenus *Nemorhina* are presented in Tables 1 and 2 for females and males, respectively. The GC profiles for males and females of each of the taxa are shown in Figures 1–4 and the major peak areas tabulated in Tables 3 and 4. The surface alkanes of these species of tsetse flies were comprised of long-chain methyl-branched alkanes with n-alkanes present as minor components. The dominant methylalkanes common to males and

TABLE 1. NORMAL AND METHYL ALKANES OF *Glossina (Glossina)*[a]

KI	Methyl-branching	G. m. centralis M	G. m. centralis F	G. m. submorsitans M	G. m. submorsitans F	G. longipalpis M	G. longipalpis F	G. swynnertoni M	G. swynnertoni F
2463	2-	–	–	+	+	–	–	+	+
2500	n-C_{25}	+	+	+	+	–	–	+	+
2533	11-; 13-	++	++	++	++	–	–	++	++
2563	11, x-	–	–	I = 3	I = 3	–	–	–	–
2572	7, x-	2-	–	+	+	+	–	+	+
2583	5, x-	–	–	I = 3/5	I = 3	I = 3/5	I = 3/5	–	–
2600	n-C_{26}	+	+	+	+	–	–	+	+
2610	5, x, y-	–	–	?+	–+	+	+	I = 3/3	I = 3, 3/3, 5?
2633	9-; 11-	–+	–+	–	++	–	–	–+	–+
	12-; 13-	–+	–+	–	++	–	–	–	–
	14-; 15-	–	–	–	+	–	–	–	–
2640	3, x, y-	t	–	–	t	–	–	–	–
2655	11, x-;	–	–	I = 3	I = 3	–	–	–	+
2665	9, x-	+	+	+	+	+	+	+	+
2672	2-	–	–	–	I = 3	–	–	–	–
2683	5, x-	+	+	t	I = 3?/5/7	+	I = 3/5/7?	+	–
2700	n-C_{27}	+	+	+	+	+	+	+	t
2720	4, x, y-	–+	–+	–+	t	–+	–+	–+	–+
2733	9-; 11-;	+	+	+	+	+	+	+	+
2739	13- 7-	–	–	–	–	–	–	–	–
2755	11, x-;	–	–	I = 3	I = 3	–	–	–	I = 3
	9, x-	+	+	+	+	+	+	+	+
2763	2-	+	+	+	+	+	+	+	+
2771	7, x-	–	–	t	I = 3/5	–	–	–	–

TABLE 1. CONTINUED

KI	Methyl-branching	G. m. centralis		G. m. submorsitans		G. longipalpis		G. swynnertoni	
		M	F	M	F	M	F	M	F
2781	5, x-	−	−	t	I = 5/7	−	I = 3	t	I = 3/5/7
2800	n-C₂₈	t	t	+	+	−	−	−	t
2833	9-; 10-;	−+	−−	−−	++	−−	−−	−−	−+
	11-; 12-;	+−	+−	+−	++	−+	+−	−−	−−
	13-; 14-	+−	+−	+−	++	+−	−−	+−	+−
2840	3, x, y-	t	t	t	I = 3, 3	t	t	t	I = 3, 3/3, 5
2863	2-	+	+	+	+	+	+	+	+
2873	7, x-	−	−	−	I = 3/5	−	−	−	−
2882	5, x-	−	−	−	I = 9?	−	−	−	−
2900	n-C₂₉	+	+	+	+	+	t	+	+
2920	4, x, y-	−	−	−	−	−	−	−	−
2933	9-; 11-;	−+	−+	−+	−+	−+	−+	−+	−+
	13-; 15-	−+	−+	++	++	++	+−	++	+−
2955	9, x-;	−	−	−	−	I = 3	−	−	−
	11, x-	−	−	−	−	−	−	−	−
2963	2-	+	+	+	+	+	+	+	+
2975	7, x-	−	−	−	I = 5	−	−	−	−
3000	n-C₃₀	t	t	+	+	−	−	−	−
3033	11-; 12-;	+−	−−	++	++	++	−+	++	−+
	13-; 14-;	+−	−−	−+	++	+−	+−	−+	−+
	15-	−	−	−	−	−	−	+	+
3040	3, x, y-	−	+	+	−	+	+	+	−
3063	2-	+	+	+	+	+	+	+	+
3100	n-C₃₁	t	t	−+	−+	−+	t	−	−
3133	9-; 11-;	−+	−+	−+	−+	−+	−+	−+	−+
	13-; 15-	+−	+−	++	++	++	−−	++	++

3163	2-	+	+	+	+		+	+
3155-	9, x-;	$I = 3$		$I = 3$				
3163	11, x-;	$I = 3$				$I = 3/9$		
	13, x-					$I = 3$		
3172	7, x-	t	t					
3180	5, x-		$I = 7$					
3170	11, x, y-	t	t	t				
3180	7, x, y-		$I = 3, 3$					
3200	n-C$_{32}$	+ +	+ +	+ +	+ +		+	+
3233	11-; 13-;		+	+		$I = 3, 3$	$I = 3, 3$	
3233	15-					$I = 3$		
3210	5, x, y-	$I = 3, 3$	$I = 3, 3$	$I = 3, 3$				
3255	10, x-;		$I = 3$			$I = 3$	$I = 3$	
	11, x-;	$I = 3$	$I = 3$					
	12, x-							
3263	2-	+	+	+	+			
3270	10, x, y-		t	t				
3280	6, x y-	+	- +					
3333	11-; 13-;	+	+	+		$I = 3/9$		
3333	15-							
3355-	11, x-;	$I = 3$	$I = 3$	$I = 3$	$I = 3$	$I = 3$		
3363	13, x-;							
3363	15, x-							
3363	2-							
3370	9, x, y-;							
3380	11, x, y-;	+	+ +	+		$I = 3, 3$	$I = 3, 3$	
3410	13, x, y		+ +			$I = 5, 3$		
3380	7, x y-							
3410	5, x y-							
3433	12-; 13-;							
3433	14-							
3455	12, x-;	$I = 3$	$I = 3$	$I = 3$	$I = 3$	$I = 9$	$I = 3$	
3455	11, x-							

TABLE 1. CONTINUED

KI	Methyl-branching	G. m. centralis		G. m. submorsitans		G. longipalpis		G. swynnertoni	
		M	F	M	F	M	F	M	F
3463	2-	+	+	–	+	–	+	–	–
3470	8, x y-	–	?	–	t	t	t	–	–
3480	6, x y-	–	–	t	–	–	–	$I = 3/?$	t
3533	13-; 15-	+–	––	?–	?–	––	++	+–	+–
3555-	11, x-;	$I = 3$	–	$I = 3$	–	$I = 3$	–	$I = 3$	$I = 3/9$
3563	13, x-;	$I = 3$	–	$I = 3$	$I = 9$	$I = 3$	$I = 3$	?	$I = 9$
	15, x-	–	$I = 3$	–	$I = 3$	–	–	–	$I = 3$
3570	11, x y-;	–	$I = 3, ?$	–	$I = 3, 3$	$I = 3, ?$	–	–	$I = 3, 3?$
	13, x, y-	–	$I = 3, 3$	$I = 3, 3$		$I = 3, ?$			
3582	7, x, y-	–	–	$I = 3, 3$	–	–	–	–	–
3610	5, x, y	–	–	$I = 5, 3$	–	–	–	–	–
3655-	12, x-;	$I = 3$	–	$I = 3$	–	$I = 3$	–	$I = 9$	$I = 9$
3663	13, x-;	–	–	–	–	–	–	–	–
	14, x-;	–	–	–	$I = 3$	$I = 3$	–	–	–
	15, x-;	$I = 3$	$I = 3$	–	$I = 3$	–	–	$I = 3$	$I = 3$
	16, x-	–	$I = 3$	–	$I = 3$	–	–	–	$I = 3$
3670	13, x, y-;	–	–	?	$I = 3, 3$	?	–	–	$I = 3, 3?$
	14, x y-	–	$I = 3, 3$	?		–	–	–	
3733	11-; 13-;	––	––	––	––	––	–+	––	––
	15-; 17-	––	––	–	––	––	++	––	––
3755-	11, x-;	–	–	$I = 3$	–	$I = 3$	–	–	–
3763	13, x-;	$I = 3$	$I = 3$	$I = 3$	$I = 3$	$I = 3$	$I = 3$	–	$I = 9$
	15, x;	$I = 3$	$I = 3$	$I = 3$	$I = 3$	–	–	–	–
	17, x-	–	–	–	–	–	–	–	~3770
	11, x, y-;	–	–	–	–	–	$I = 3, ?$	–	–

3790	13, x, y-	—	—	—	—	?	—	—
	15, x, y-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	—	—
	11, x, y	—	—	I = 3, 3, 3	I = 3, 3, 3	—	—	I = 3, 3, 3
	z-							
3855-	12, x-	—	—	—	—	—	—	I = 9
3863	15, x-	I = 3	I = 3	I = 3	I = 3	—	—	I = 3
	16, x-	I = 3	I = 3	—	I = 3	—	—	I = 3
	17, x-	—	—	—	—	—	—	I = 3
3870	15, x, y-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	—	—
	16, x, y-	I = 3, 3	—	—	—	—	—	I = 3, 3
3955-	13, x-	—	—	—	—	—	—	I = 9/11?
3963	15, x-	I = 3	—	I = 3	I = 3	—	—	—
3970	15, x, y-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	t	—	I = 3, 3

ᵃ +, isomer present; —, isomer not present at detectable levels; t, peak present but identification of isomers ambiguous; ?, methyl-branching ambiguous. Each mark represents one compound or one isomer. I = number of methylene carbons between branches.

ᵇ x, position of the second methyl branch; y, position of the third methyl branch.

TABLE 2. NORMAL AND METHYL ALKANES OF Glossina (Nemorhina)[a]

KI	Methyl branching	G. f. martinii		G. caliginea		G. p. pallicera		G. p. newsteadi	
		M	F	M	F	M	F	M	F
2500	$n\text{-}C_{25}$	+	+	+	+	+	+	+	+
2563	2-	+	+	+	+	+	+	+	+
2600	$n\text{-}C_{26}$	+	+	+	+	+	+	+	+
2640	3, x, y-	I = 3, 3	I = 3, 3	-	-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3
2663	2-	+	+	+	+	+	+	+	+
2700	$n\text{-}C_{27}$	+	+	+	+	+	+	+	+
2720	4, x, y-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3
2763	2-	+	+	+	t	+	+	t	+
2800	$n\text{-}C_{28}$	t	t	+	t	+	+	t	t
2810	5, x, y-	-	-	-	-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3
2840	3, x, y-	I = 3, 3; 3, 5	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3
2863	2-	+	+	+	+	+	+	+	+
2900	$n\text{-}C_{29}$	-	-	+	t	+	+	+	+
2920	4, x, y-	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3	I = 3, 3
2963	2-	+	+	+	+	+	+	+	+
3000	$n\text{-}C_{30}$	-	-	+	-	-	-	-	-
3010	5, x, y	I = 3, 3	I = 3, 3	I = 3, 3; 3, 5	-	-	-	-	-
3040	3, x, y-	I = 3, 3	I = 3, 3	I = 3, 3; 3, 5	-	-	-	-	-

		1	2	3	4	5	6	7	8
3063	2-	+	+	+	+	+	+	+	+
3100	*n*-C$_{31}$	−	−	−	−	−	−	−	−
3155	13, *x*-;	−	−	−	−	−	$I = 3$	−	−
	11, *x*-;	+	+	+	+	+	$I = 3$	+	+
3163	2-	+	+	+	+	−	?	−	−
3170	11, *x*, *y*-	−	−	−	$I = 3$	−	$I = 3, 3$	−	−
3263	2-	−	−	−	+	−	?	−	−
3255	10, *x*-;	−	−	−	−	−	$I = 3$	$I = ?$	$I = 3$
	12, *x*-	−	−	−	?	−	$I = 3$	$I = ?$	$I = 3$
3271	10, *x*, *y*-;	$I = 3, 3?$	$I = 3, 3?$	−	−	−	$I = 3, 3$	−	−
	12, *x*, *y*-	$I = 3$	$I = 3$	−	−	−	$I = 3, 3$	−	−
3355	11, *x*-;	$I = 3$	$I = 3$	−	$I = 3$	−	$I = 3$	$I = 3$	$I = 3$
	13, *x*-;	$I = 3$	$I = 3$	−	$I = 3$	−	$I = 3$	$I = 3$	$I = 3$
	15, *x*-	−	−	−	?	−	−	−	−
3363	2-	+	+	−	$I = 3, 3$	−	−	−	−
3370	11, *x*, *y*-	$I = 3, 3?$	$I = 3, 3?$	−	?	−	$I = 3, 3$	$I = 3, 3$	$I = 3, 3$
3455	12, *x*-;	$I = 3$	$I = 3$	−	−	−	t	−	t
	10, *x*-	−	−	−	−	−	t	−	?
3470	12, *x*, *y*-	$I = 3, 3?$	$I = 3, 3?$	−	−	−	−	$I = 3, 3$	$I = 3, 3$
3555	11, *x*-;	$I = 3$	$I = 3$	−	−	−	t	$I = 3$	$I = 3$
	13, *x*-	$I = 3$	$I = 3$	−	−	−	t	$I = 3$	$I = 3$
3570	11, *x*, *y*;	$I = 3, 3?$	$I = 3, 3?$	−	$I = 3, 3$	−	−	$I = 3, 3$	$I = 3, 3$
	13, *x*, *y*-	$I = 3, 3?$	$I = 3, 3?$	−	$I = 3, 3$	−	−	−	−

ᵃ +, isomer present; −, isomer not present at detectable levels; t, peak present at trace levels but identification of isomers ambiguous; ?, methyl-branching ambiguous; *x*, *y*, and *I* as in Table 1.
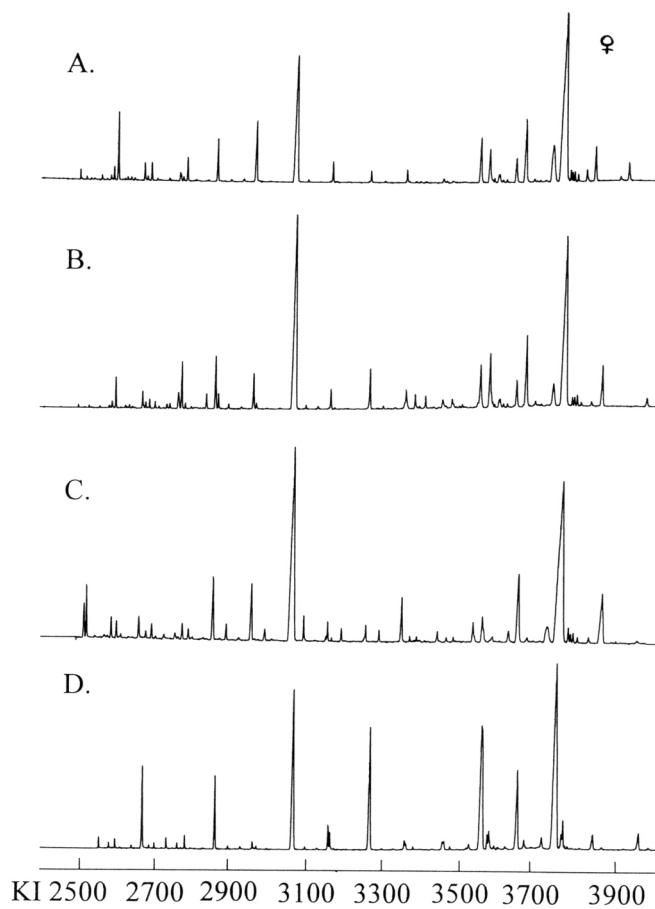
FIG. 1. GC of females *Glossina* (*Glossina*) cuticular alkanes: (A) *G. morsitans centralis*;
(B) *G. m. submorsitans*; (C) *G. swynnertoni*; (D) *G. longipalpalis*.

females in both subgenera consisted of an odd-carbon-number backbone series
of 2-methylalkanes having modes at 29 or 31 carbons, and a homologous even-
carbon-number backbone series with a mode at 30 carbons. Internally branched
monomethylalkanes were present at relatively low levels with the 15-, 13-, and
11- isomers coeluting at Kovats index (Kovats, 1965) KI $XX28$–$XX33$, where
XX refers to the first two digits of the KI number, and internal branching refers
to branch positions near the center of the carbon chain. There was no un-
ambiguous evidence for the presence of 3- or 4-methylalkanes in any of the taxa
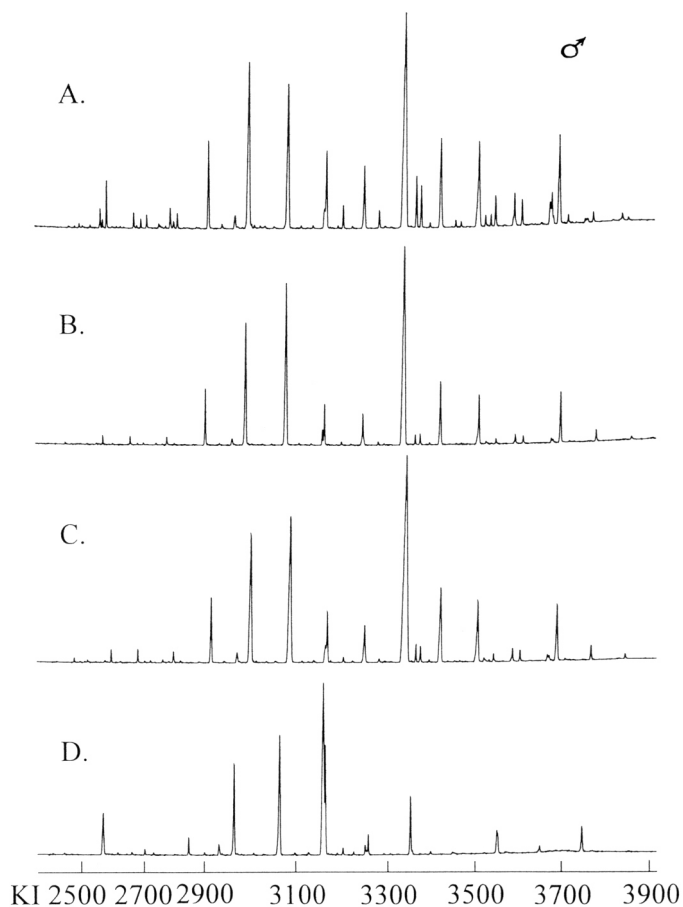
Fig. 2. GC of male *Glossina* (*Glossina*) cuticular alkanes: (A) *G. morsitans centralis*; (B) *G. m. submorsitans*; (C) *G. swynnertoni*; (D) *G. longipalpalis*.

using either the 30-m or 50-m column, the latter producing about 300,000 theoretical plates.

Adult females were characterized by the presence of relatively large quantities of internally branched di- and/or trimethylalkanes in addition to the 2-methylalkanes. These compounds were present in the males, if detected, in significantly smaller quantities. Internally branched tetramethylalkanes were also present albeit at relatively low levels.

Subgenus Glossina. The methylalkanes of *G. morsitans centralis* and
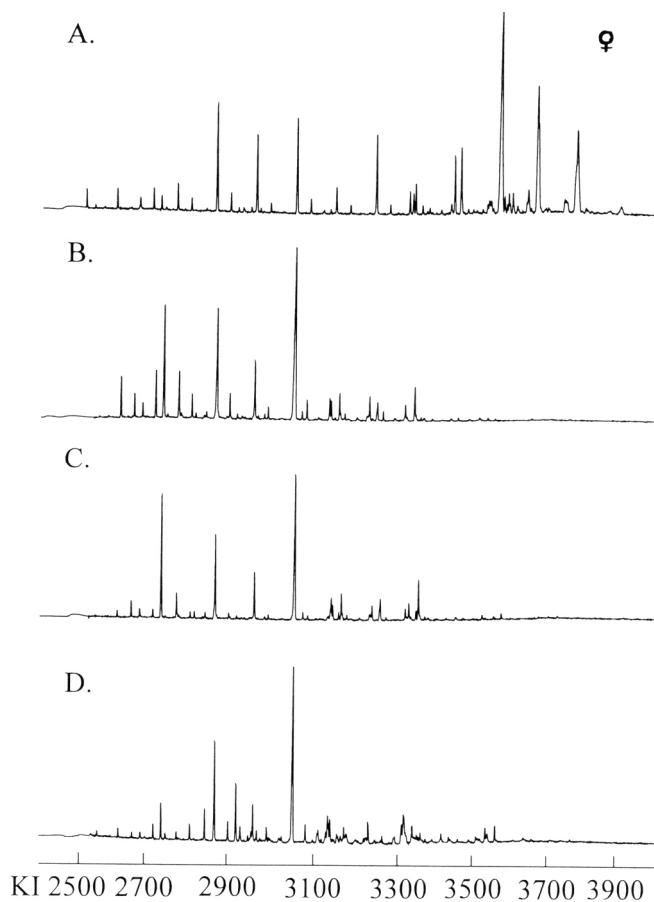
FIG. 3. GC of female *Glossina* (*Nemorhina*) cuticular alkanes: (A) *G. fuscipes martinii*; (B) *G. pallicera pallicera*; (C) *G. p. newsteadi*; (D) *G. caliginea*.

G. m. submorsitans were essentially identical (Figure 1), being dominated by a peak at KI 3770 (37.6% and 33.7% of total, respectively). EI mass spectra of this peak showed major fragments at m/z 224/225, 295, and 365. The 224/225 doublet with m/z 224 > 225 indicated cleavage internal to the methyl branch that is closest to the end of the chain, and the lack of a second even/odd doublet showed that the compound is symmetric. Minor fragments at m/z 196/197 (in part), 266/267, and 336/337 were of a magnitude consistent with primary carbonium ions of the m/z 224/225, 295, and 365 secondary ions, respectively,
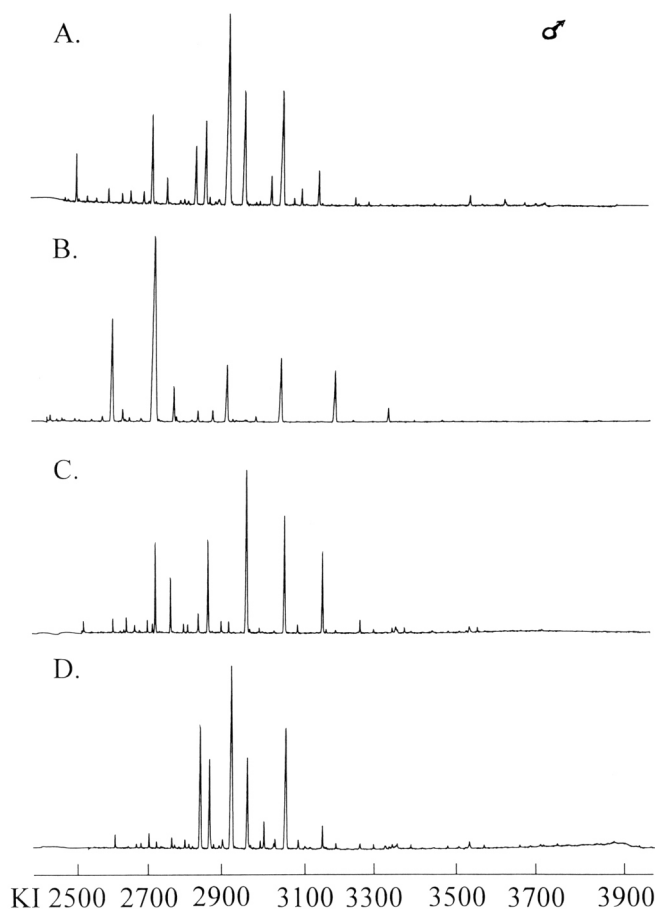
FIG. 4. GC of male *Glossina* (*Nemorhina*) cuticular alkanes: (A) *G. fuscipes martinii*; (B) *G. pallicera pallicera*; (C) *G. p. newsteadi*; (D) *G. caliginea*.

and did not necessarily indicate the presence of additional isomers. This fragmentation pattern was consistent with 15,19,23-trimethylheptatriacontane, having a pattern of methylene interruptions (*I*) between the branch points of the form *I* = 3,3. Both EI mass spectra and KI were found to be identical with both the synthetic and the natural compound originally identified from *G. morsitans morsitans* (Carlson et al., 1978). The $[M-15]^+$ fragment at *m/z* 547 was not identifiable in the EI spectra but CI-MS confirmed the presence of 40 carbons. Homologs of 15,19,23-trimethylheptatriacontane were also present with

TABLE 3. ALKANES OF G. (Glossina) AND G. (Nemorhina) FEMALES

Percent of total peak area (mean ± SD)[b]

Type[a]	KI	G.m.c.	G.m.s.	G.s.	G.l.	G.f.m.	G.p.p.	G.p.n.	G.c.
26	2600	0.1 ± 0.0	0.2 ± 0.2	1.2 ± 0.7	0.4 ± 0.4	0.4 ± 0.3	1.6 ± 1.2	0.6 ± 0.1	0.9 ± 0.5
26A	2633	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		0.2 ± 0.1	0.5 ± 0.6	0.1 ± 0.1
25C'	2640		0.1 ± 0.1			0.7 ± 0.2	1.6 ± 0.1	1.8 ± 0.5	0.3 ± 0.1
26A'	2663	1.1 ± 0.4	0.8 ± 0.2	0.8 ± 0.3	1.7 ± 1.4	0.5 ± 0.1	1.2 ± 0.3	0.6 ± 0.1	0.8 ± 0.4
27	2700	0.1 ± 0.0	0.3 ± 0.2	0.4 ± 0.4	0.5 ± 0.6	1.0 ± 0.4	2.2 ± 1.5	0.7 ± 0.0	1.1 ± 0.2
26C'	2720		0.1 ± 0.0		0.1 ± 0.1		10.3 ± 0.9	12.2 ± 2.7	1.7 ± 1.2
27A	2733	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.4 ± 0.2
27A'	2763	1.3 ± 0.5	0.9 ± 0.4	0.3 ± 0.1	0.6 ± 0.3	0.5 ± 0.3	3.2 ± 0.9	2.3 ± 1.2	0.6 ± 0.2
28A	2833	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
27C'	2840	0.0 ± 0.1	0.4 ± 0.3	0.1 ± 0.0		0.1 ± 0.1	0.4 ± 0.1	1.1 ± 0.5	3.4 ± 1.8
28A'	2863	1.9 ± 0.8	2.0 ± 0.9	2.7 ± 1.3	2.1 ± 0.9	5.0 ± 2.3	14.3 ± 4.3	7.1 ± 5.7	10.4 ± 3.5
28C'	2920					0.1 ± 0.1	0.5 ± 0.4	0.6 ± 0.5	5.7 ± 1.7
29A	2933	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.7	0.2 ± 0.0	1.1 ± 0.5
29A'	2963	3.7 ± 0.9	1.9 ± 0.6	4.5 ± 1.3	1.0 ± 0.6	3.5 ± 0.9	4.9 ± 1.0	5.3 ± 1.0	4.1 ± 0.3
30A	3033		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.4	0.1 ± 0.0	1.1 ± 0.4
29C'	3040					0.2 ± 0.1	0.9 ± 1.2	0.3 ± 0.1	0.2 ± 0.2
30A'	3063	19.6 ± 2.2	23.1 ± 5.9	26.9 ± 0.4	10.4 ± 2.7	4.7 ± 3.5	26.1 ± 6.5	18.3 ± 9.3	21.3 ± 0.4
31A	3133		0.1 ± 0.1		0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	2.2 ± 0.1
31B	3155	1.5 ± 0.5	1.4 ± 0.7	2.2 ± 1.7	4.5 ± 3.7	1.1 ± 0.5	5.0 ± 3.3	5.5 ± 1.2	7.8 ± 0.4
31C	3170	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		6.3 ± 3.3	2.4 ± 3.2	5.8 ± 0.8
32A	3233		0.1 ± 0.0		0.1 ± 0.1		0.6 ± 0.3	0.3 ± 0.2	1.0 ± 0.6
32B	3255	1.3 ± 0.8	3.1 ± 0.5	1.5 ± 0.8	8.5 ± 0.8	0.1 ± 0.1	2.5 ± 0.8	5.8 ± 3.6	3.8 ± 0.2
32C	3270		0.1 ± 0.0			3.9 ± 1.1	3.1 ± 0.8	2.0 ± 2.6	2.2 ± 0.3
33A	3333		0.1 ± 0.1		0.2 ± 0.1	0.1 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	1.0 ± 0.4
33B	3355	0.8 ± 0.1	1.7 ± 1.1	7.4 ± 6.5	1.6 ± 0.7	3.5 ± 1.9	3.4 ± 1.2	12.8 ± 11.5	8.9 ± 0.4
33C	3570		0.6 ± 0.4		0.1 ± 0.1	0.5 ± 0.2	6.3 ± 7.1	6.6 ± 0.5	5.0 ± 0.6

34A	3433	0.1 ± 0.0			0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1		0.5 ± 0.2
34B	3455	0.8 ± 0.4	0.9 ± 0.1	1.6 ± 1.2	1.4 ± 0.3	3.9 ± 1.3	0.5 ± 0.2	2.0 ± 1.9		0.8 ± 0.1
34C	3470	0.2 ± 0.0	0.7 ± 0.4	0.2 ± 0.2	0.3 ± 0.1	4.1 ± 1.1	0.3 ± 0.1	0.7 ± 0.0		0.9 ± 0.0
35A	3533		0.1 ± 0.1		0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2		0.2 ± 0.1
35B	3555	4.1 ± 0.1	3.4 ± 0.8	2.6 ± 1.6	19.0 ± 1.7	2.7 ± 0.9	0.4 ± 0.3	4.4 ± 4.9		1.5 ± 0.5
35C	3570	3.2 ± 0.0	4.5 ± 2.0	1.8 ± 0.7	2.8 ± 0.7	24.4 ± 8.7	0.6 ± 0.4	2.8 ± 3.3		2.4 ± 0.4
35D	3590	0.2 ± 0.0	1.1 ± 0.5		0.3 ± 0.0	2.0 ± 1.1	0.0 ± 0.1	0.1 ± 0.0		0.7 ± 0.9
36A	3633		0.1 ± 0.1		0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1		0.1 ± 0.2
36B	3655	2.2 ± 0.0	1.8 ± 0.2	1.1 ± 0.3	6.6 ± 1.0	2.6 ± 0.9	0.1 ± 0.1	0.4 ± 0.3		0.1 ± 0.1
36C	3670	6.3 ± 0.3	5.7 ± 0.2	3.9 ± 2.9	0.7 ± 0.1	14.3 ± 3.1	0.1 ± 0.1	0.2 ± 0.1		0.4 ± 0.6
36D	3690	0.1 ± 0.0	0.4 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.7 ± 0.2		0.1 ± 0.1		0.1 ± 0.2
37A	3733	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.2	0.1 ± 0.0		0.1 ± 0.1		0.1 ± 0.2
37B	3755	6.6 ± 0.1	3.0 ± 0.8	3.4 ± 0.9	27.5 ± 4.1	2.3 ± 0.8	0.1 ± 0.1	0.4 ± 0.3		0.3 ± 0.4
37C	3770	37.6 ± 0.3	33.7 ± 4.4	27.4 ± 9.9	2.6 ± 0.3	12.3 ± 3.5		0.2 ± 0.2		0.2 ± 0.3
37D	3790	0.9 ± 0.1	0.8 ± 0.4	0.7 ± 0.4	0.2 ± 0.0	0.6 ± 0.1		0.1 ± 0.1		0.1 ± 0.1
38A	3833		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1				
38B	3855	0.9 ± 0.0	0.5 ± 0.3	0.4 ± 0.0	1.3 ± 0.3	0.7 ± 0.5				0.1 ± 0.2
38C	3870	2.7 ± 0.3	4.2 ± 1.2	5.4 ± 1.0	0.2 ± 0.0	1.2 ± 0.1				
38D	3890	0.0 ± 0.1	0.1 ± 0.1			0.1 ± 0.1				
39A	3933				0.1 ± 0.0					0.1 ± 0.1
39B	3955	0.3 ± 0.0	0.3 ± 0.2	0.3 ± 0.2	1.7 ± 0.4					0.1 ± 0.2
39C	3970	1.2 ± 0.2	0.9 ± 0.6	1.7 ± 2.1	0.2 ± 0.0	0.2 ± 0.1			0.2 ± 0.1	0.2 ± 0.2
39D	3990					0.5 ± 0.3				

[a] A = internal methyl, B = internal dimethyl, C = internal trimethyl, D = internal tetramethyl, A' = 2-methyl, C' = 3, 7, 11- or 4, 8, 12-trimethyl alkanes.

[b] *G. m. centralis* (G.m.c.), *G. m. submorsitans* (G.m.s.), *G. swynnertoni* (G.s.), *G. longipalpis* (G.l.), *G. f. martinii* (G.f.m.), *G. p. pallicera* (G.p.p.), *G. p. newsteadi* (G.p.n.), *G. caliginea* (G.c.).

TABLE 4. ALKANES OF *G. (Glossina)* AND *G. (Nemorhina)* MALES

Type[a]	KI	G.m.c.	G.m.s.	G.s.	G.l.	G.f.m.	G.p.p.	G.p.n.	G.c.
				Percent of total peak area (mean ± SD)[b]					
26	2600	0.1 ± 0.0	0.3 ± 0.2		0.3 ± 0.2	0.7 ± 0.1	0.8 ± 0.3	0.9 ± 0.5	0.4 ± 0.3
26A	2633	0.1 ± 0.0	0.1 ± 0.0			0.1 ± 0.1	0.1 ± 0.0	1.0 ± 1.4	0.1 ± 0.1
25C'	2640	0.1 ± 0.0				1.6 ± 1.9	7.6 ± 6.7	1.2 ± 0.9	
26A'	2663	0.5 ± 0.0	0.5 ± 0.3	0.5 ± 0.0	2.4 ± 2.2	0.7 ± 0.1	1.2 ± 0.6	0.4 ± 0.3	0.5 ± 0.4
27	2700	0.3 ± 0.2	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.2	0.8 ± 0.0	0.9 ± 0.5	0.8 ± 0.4	1.0 ± 0.6
26C'	2720			0.1 ± 0.1	0.1 ± 0.0	14.5 ± 12.7	36.8 ± 11.5	10.6 ± 2.1	0.9 ± 0.6
27A	2733	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
27A'	2763	0.8 ± 0.1	1.0 + 0.5	0.5 ± 0.0	0.5 ± 0.3	1.8 ± 0.5	4.4 ± 1.7	4.3 ± 1.3	0.7 ± 0.1
28A	2833	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0				
27C'	2840		0.2 ± 0.2			5.2 ± 0.3	0.9 ± 0.3	1.7 ± 0.3	17.5 ± 3.5
28A'	2863	3.9 ± 0.3	1.8 ± 0.3	3.7 ± 0.2	1.4 ± 0.5	7.2 ± 1.9	10.1 ± 3.1	7.7 ± 5.0	10.0 ± 1.1
28C'	2920					22.0 ± 12.4	0.6 + 0.3	0.9 ± 0.3	33.6 ± 3.9
29A	2933	0.6 ± 0.5	0.3 ± 0.2	0.8 ± 0.1	1.4 ± 0.4		0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
29A'	2963	14.0 ± 0.1	12.8 ± 8.8	12.2 ± 0.2	6.7 ± 2.8	13.5 ± 3.2	17.7 ± 6.6	31.2 ± 0.7	6.9 ± 3.7
30A	3033	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1		0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2
29C'	3040					2.9 ± 2.1	0.2 ± 0.2	0.3 ± 0.1	0.6 ± 0.2
30A'	3063	14.1 ± 3.3	21.7 ± 9.0	18.5 ± 1.8	19.1 ± 1.3	16.8 ± 6.6	10.3 ± 2.7	14.6 ± 4.4	4.7 ± 3.5
31A	3033	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.3		0.2 ± 0.1	0.1 ± 0.2	0.4 ± 0.2
31B	3055	6.4 ± 0.8	8.0 ± 4.1	4.1 ± 0.1	29.6 ± 12.1	3.3 ± 0.8	1.9 ± 0.4	12.0 ± 2.7	1.6 ± 0.4
31C	3070	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
32A	3233	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
32B	3255	4.3 ± 0.6	4.9 ± 1.7	2.8 ± 0.1	5.5 ± 1.7	0.5 ± 0.2	0.4 ± 0.2	1.1 ± 0.1	0.6 ± 0.2
32C	3270		0.5 ± 0.7		0.0 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.7

		G.m.c.	G.m.s.	G.s.	G.l.	G.f.m.	G.p.p.	G.p.n.	G.c.
33A	3333	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	0.4 ± 0.3	0.2 ± 0.2	0.2 ± 0.1	0.6 ± 0.4
33B	3355	29.7 ± 4.1	29.3 ± 1.3	35.6 ± 1.1	19.2 ± 11.4	0.2 ± 0.3	0.5 ± 0.4	1.7 ± 0.6	1.3 ± 1.0
33C	3370	0.1 ± 0.0	1.3 ± 1.6		0.1 ± 0.0		0.4 ± 0.3	1.5 ± 1.1	0.9 ± 0.4
34A	3433	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.1		0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
34B	3455	6.7 ± 0.4	4.4 ± 0.8	6.5 ± 0.4	1.2 ± 0.6	0.5 ± 0.5	0.2 ± 0.2	0.5 ± 0.3	0.3 ± 0.2
34C	3470		1.5 ± 2.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.1 ± 0.1	0.6 ± 0.7	0.7 ± 0.4
35A	3533	0.1 ± 0.0	0.2 ± 0.1		0.2 ± 0.0	0.1 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.3
35B	3555	6.0 ± 1.5	3.2 ± 0.3	5.0 ± 0.3	4.7 ± 1.4	0.3 ± 0.1	0.5 ± 0.5	1.3 ± 0.1	1.1 ± 0.3
35C	3570	0.3 ± 0.1	0.4 ± 0.3	0.4 ± 0.0	0.3 ± 0.1	1.7 ± 1.1	0.4 ± 0.4	1.7 ± 1.5	0.7 ± 1.0
35D	3590	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 1.1	0.1 ± 0.2	0.1 ± 0.0		
36A	3633	0.1 ± 0.0	0.0 ± 0.1		0.3 ± 0.5		0.2 ± 0.1		0.1 ± 0.1
36B	3655	1.5 ± 0.5	0.4 ± 0.0	0.8 ± 0.0	0.8 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.0	0.2 ± 0.2
36C	3670	0.7 ± 0.5	0.6 ± 0.4	0.6 ± 0.0	0.1 ± 0.1	1.0 ± 0.4	0.1 ± 0.1	0.4 ± 0.3	0.2 ± 0.3
36D	3690		0.1 ± 0.1			0.1 ± 0.0	0.1 ± 0.1		
37A	3733	0.2 ± 0.0	0.1 ± 0.1		0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.4
37B	3755	2.5 ± 1.2	0.8 ± 0.4	0.8 ± 0.0	2.8 ± 1.4	0.5 ± 0.2	0.4 ± 0.3	0.4 ± 0.1	0.7 ± 0.6
37C	3770	4.0 ± 3.3	2.7 ± 0.2	4.5 ± 0.0	0.2 ± 0.1	0.9 ± 0.5	0.5 ± 0.4	0.6 ± 0.8	0.6 ± 0.6
37D	3790					0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	
38A	3833	0.1 ± 0.0	0.1 ± 0.1			0.2 ± 0.4	0.1 ± 0.1	0.1 ± 0.0	
38B	3855	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
38C	3870	0.3 ± 0.3	0.3 ± 0.1	0.8 ± 0.1		0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
38D	3890					0.1 ± 0.0	0.0 ± 0.1		
39A	3933		0.1 ± 0.1			0.1 ± 0.1			
39B	3955	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0		0.2 ± 0.1		0.1 ± 0.1	0.1 ± 0.2
39C	3970	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.0		0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.2
39D	3990	0.1 ± 0.1				0.1 ± 0.1		0.1 ± 0.1	

backbone carbon numbers of 38 and 39. In addition, smaller quantities of other trimethylalkane isomers were present with methyl branching ranging from 3,x,y- to 9,x,y- and 13,x,y- to 14,x,y-, where x refers to the position of the second methyl group, and y refers to the position of the third methyl group. The 3,x,y- the 7,x,y- isomers were the predominant trimethylalkanes at backbone carbon numbers below 35, with much less of the even backbone homologs. Dimethylalkanes were present at relatively low levels with methyl branching from 5,x- to 17,x-. The internally branched isomers, 11,x- to 17,x-, coeluted, forming a homologous series of peaks at KI XX55 and were quantitatively correlated with the 15,19,23- series. The CI-MS confirmed the presence of small quantities of tetramethylalkanes at KI XX90 with the mode at KI 3790, and the EI spectra for the compound at KI 3790 was consistent with a single isomer, 11,15,19,23-tetramethylheptatriacontane.

In adult males of G. m. centralis and G. m. submorsitans, 15,19,23-tri-methylheptatriacontane was present only as a minor component (4 and 2.7%, respectively), whereas the major peak (29 and 29%) was identified as 11,15-dimethyltritriacontane (KI 3355). This compound was also present in adult females (range 0.8-1.7%).

Both males and females of G. swynnertoni also exhibited methylalkane patterns within the range of variation seen in the G. morsitans subspecies (Figures 1 and 2). EI mass spectra of the predominant peak in adult females (27.4%) at KI 3770 showed major fragments as above for 15,19,23-trimethylheptatriacontane. Hydrocarbon patterns and composition of adult males of G. sywnnertoni were essentially identical with G. morsitans males.

Female G. longipalpalis exhibited a pattern in which trimethylalkanes, although present, were not dominant. In this species the predominant peak was found to vary between KI 3563 and KI 3763, depending on the individual (19.0 and 27.5%, respectively). EI mass spectra of the peak at KI 3563 (Figure 5a) showed major fragments m/z 168/169, 196/197, 224/225, 323, 351, 379, and 505 [M−15]$^{+}$. Examination of successive scans across the KI 3563 peak for each of the G. longipalpis females indicated that the doublet at m/z 196/197 was strongly correlated with the fragment at 351 and that the remaining fragments were associated with another dimethyl isomer. The m/z 196/197 and 351 fragments were consistent with 13,23-dimethylpentatriacontane, which has an interruption of nine methylenes between methyl branches (I = 9). The remaining fragments were consistent with the 11,21- homolog. The EI spectra and KI of this peak were consistent with that reported for natural and synthetic 11,21- and 13,23-dimethylpentatriacontane in G. pallidipes (Carlson et al., 1984). In all specimens examined, 13,23-dimethylpentatriacontane was the dominant isomer at KI 3563 with the ratio of the abundances of the m/z 351 and 379 fragments greater than 2:1. The EI mass spectra of the apex of the peak at KI 3763 (Figure 5b) showed major fragments at m/z 196/197, 224/225, 351, 379, and 533
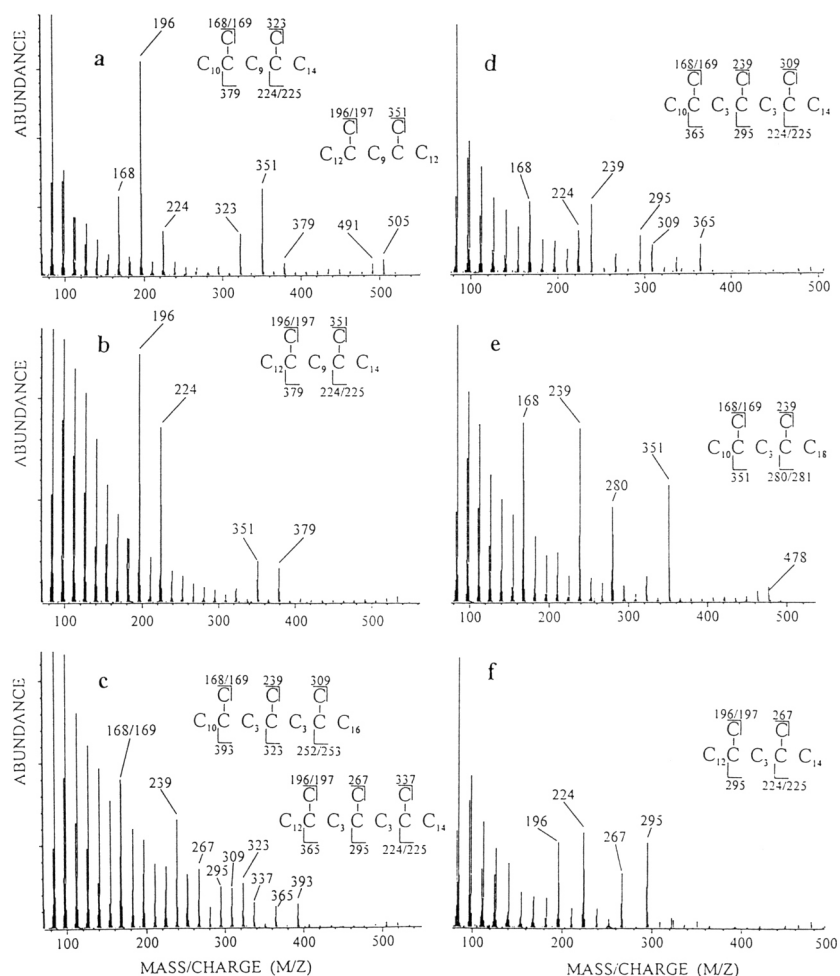
FIG. 5. GC-MS spectra of apex of GC-MS peaks at: (a) KI 3563, *G. longipalpis* female; (b) KI 3763, *G. longipalpis* female; (c) KI 3570, *G. f. martinii* female; (d) KI 3370, *G. p. pallicera* female; (e) KI 3355, *G. p. pallicera* female (*m/z* 477 ion recorded as 478 due to mass defect); (f) KI 3153, *G. caliginea* female.

$[M-15]^+$, consistent with 13,23-dimethylheptatriacontane ($I = 9$); this molecular weight was consequently confirmed by CI, and no other isomers were detected. The EI scans at the front of the peak at KI 3563 suggested the presence of additional isomers of 11,*x*-, 13,*x*-, and/or 15,*x*-dimethylalkanes with $I = 3$. Dimethylalkane isomers with three methylene units between methyl positions

were identified ranging in backbone carbon number from 27 to 38, with the series mode at 31 carbons. The KI 3155 peak consisted of 11,*x*- and 13,*x*-dimethylalkanes having $I = 3$ and was partially resolved from the corresponding isomers having $I = 9$ eluting at KI 3163. Dimethylalkanes having intermediate numbers of methylene units (5- or 7-) were not identified. Internally branched trimethylalkanes of the form 11,15,*y*-, 13,17,*y*-, and/or 15,19,*y*- were also present at KI *XX*70 with the largest peaks at KI 3570 and KI 3770. Given the complexity of the EI spectra as a result of the presence of multiple trimethylalkane isomers and the paucity of fragments, it was not possible to reliably reconstruct the more internal methyl positions; however, the fragments suggest that more than three methylene units are present between the two most interior methyl branches in at least some isomers. Other trimethylalkanes were present with backbone carbon numbers below 33. Tetramethylalkanes having a 37-carbon backbone were present at KI 3790.

Males of *G. longipalpis* were found to have negligible quantities of dimethylalkanes having nine methylene units between methyl branches. The predominant internally branched methyl-branched alkane in males was identified as 11,15-dimethyltritriacontane (29.6%).

Subgenus Nemorhina. Females of *G. f. martinii* were found to possess, in addition to the 2-methylalkane series, a major peak eluting at KI 3570 (Figure 3). The CI mass spectra indicated the presence of 38 carbons, and the EI mass spectra (Figure 5c) showed a major doublet at *m/z* 168/169 with 168 > 169 and the next significant fragment at *m/z* 239. This was consistent with one methyl on position 11 and a second methyl at position 15. The other significant ions that could result from fragmentation of a 11,15,*y*-trimethylalkane were at *m/z* 309, 337, 365, and 393. For $I = 3,3$, the *m/z* 309 fragment would result from the third methyl at position 19. The remaining fragments at *m/z* 393, 323, and 252/253 were consistent with a 11,15,19-trimethylalkane having a 35-carbon backbone as identified as the minor isomer in *G. p. palpalis* (Nelson et al., 1988). The 252/253 doublet did show 252 > 253 as consistent with a terminal fragment (one that had contained only one methyl branch); however the abundance of this ion relative to *m/z* 323 is low. This could be an artifact resulting from background subtraction. The remaining fragments were consistent with the 13,17,21- isomer, although the 196/197 doublet is not evident. Alternatively, isomers having five or more methyl units between the more interior methyl branches may be present. The strong ions at *m/z* 168/169, 239, 323, and 393 suggest that the dominant isomer is 11,15,*x*-trimethylpentatriacontane; however, the exact branching sequence remains questionable. Dimethylalkanes, predominantly 11,15- and 13,17-isomers, were also present at low levels in females of *G. f. martinii*.

The methylalkane patterns of adult *G. fuscipes martinii* males (Figure 4) were dominated by peaks at KI 2720 or KI 2920 (30–60% of total), depending

upon the individual, in addition to the homologous series of 2-methylalkanes. The EI mass spectra of the peak at KI 2920, the predominant peak in most individuals, showed major fragments at m/z 141, 211, 252/253, 323, and 393. Although the doublet at m/z 70/71 expected with EI fragmentation of 4,8,12-trimethyloctacosane was not distinguishable from the alkyl background, the remaining fragments and the CI mass spectra were consistent with this structure. There was no evidence to support the presence of other isomers in this peak. Similarly, EI mass spectra of the peak at KI 2720 showed major ions at m/z 141, 211, 224/225, 295, and 365 consistent with 4,8,12-trimethylhexacosane, the KI 2920 homolog. The homologous 3,7,11-trimethylpentacosane (KI 2640), heptacosane (KI 2840), and nonacosane (KI 3040) were present in smaller quantities. These trimethylalkanes were only found as minor components in the adult females of *G. f. martinii.*

The females of both subspecies of *G. pallicera* exhibited variability in the relative abundances of long-chain internally branched methylalkanes (<5% to 15%). EI scans of the predominant peak (KI 3370) in most individuals examined (Figure 5d) showed major fragments at m/z 168/169, 224/225, 239, 295, 309, and 365 consistent with a 11,15,19-trimethylalkane having a 33-carbon backbone. Homologs were present at KI 3170 and KI 3570. In one adult female of *G. p. newsteadi* the corresponding dimethylalkane series was predominant. EI scans of the mode of this series at KI 3355 (Figure 5e) showed major fragments m/z 168/169, 239, 280/281, 351, and 477 [M − 15]⁺. This was consistent with 11,15-dimethyltritriacontane, the dimethyl analog of the predominant trimethylalkane isomer.

G. pallicera males of both subspecies had methylalkane compositions similar to that of *G. f. martinii* (Table 1). The 4,8,12-trimethylalkane and 2-methylalkanes series had modes at 27 and 29/30 carbons, respectively.

Internally branched alkanes were present as minor constituents in the two adult females of *G. caliginea* analyzed (Figure 3). In both sexes the methylalkanes were dominated by 3,7,11-/4,8,12-trimethylalkanes and 2-methylalkanes, with the former predominant in the males. EI mass spectra of the peak at KI 3153 in the females (Figure 5f) showed major fragments at m/z 196/197, 224/225, 267, and 295, consistent with a 13,17-dimethylalkane having a backbone carbon number of 31. The corresponding peak at KI 3355 showed major fragments at m/z 224/225 and 295 together with less abundant fragments at 196/197, 252/253, 267, and 323. A series of scans across this peak indicated that the former fragments were strongly correlated. This is consistent with a symmetric 15,19-dimethylalkane having a 33-carbon backbone as identified in males of *G. m. morsitans* (Nelson and Carlson, 1986). The remaining fragments were also correlated and represent an isomer eluting slightly later than the 15,19-dimethylalkane. That fragmentation was interpreted as the 33-carbon backbone homolog of the 13,17-dimethylalkane at KI 3155. A second homologous series

of internally branched alkanes (KI 3170 to KI 3570) was also present in the cuticular extracts of adult females of G. caliginea. EI mass spectra of the series mode at KI 3370 showed major fragments at 168/169, 224/225, 239, 295, 309, and 365, and the CI mass spectra indicated the presence of 36 carbons. This was consistent with a 33-carbon backbone trimethylalkane having an 11,15,19-structure. The corresponding 31-carbon backbone homolog was identified at KI 3155. EI mass spectra of the remaining significant peak in this series at KI 3570 showed major fragments at m/z 196/197, 224/225, 267, 295, 337, and 365 and was identified as 13,17,21-trimethylpentatriacontane. Dimethylalkanes and trimethylalkanes were also present in adult males of G. caliginea albeit at low levels. The mode for the 4,8,12-trimethylalkane series in G. caliginea males was at 29 carbons in the two individuals analyzed and the mode for the 2-methylalkane series at 30 carbons.

DISCUSSION

Tsetse flies of the subgenera Glossina and Nemorhina exhibit common patterns of cuticular alkanes consisting of even- and odd-carbon-number backbone homologous series of 2-methylalkanes, low levels of n-alkanes and, especially in females, long-chain internally branched di- and trimethylalkanes having backbone carbon numbers in the range of 31–39 carbons. Adults of both subgenera exhibit sexual dimorphism in the relative proportion of the internally branched methylalkanes, with the longer di- and/or trimethyl isomers often being the predominant saturated hydrocarbon compounds in females, and are implicated as mediators of sexual response in males of several species.

The subgenus Nemorhina shows a homologous series of 3,7,11- (KI XX40, exclusively odd-carbon-number backbone) and 4,8,12-trimethylalkanes (KI XX20, exclusively even-carbon-number backbone). The relative proportion of these compounds is sexually dimorphic in adults, with the 4,8,12- series of isomers the predominant methylalkanes in most males. Mass spectra for both series of trimethylalkanes were described from G. palpalis palpalis males (Nelson et al., 1988). The biosynthetic pathway is probably similar for this series, as they often appear together, and the remainder of the molecule is the same after the first branch point in an odd-/even-carbon-number backbone pair. The first published examples of trimethyls with both branching patterns were larger C_{34}–C_{39} alkanes found in ants (Martin and MacConnell, 1970), whereas only 3,7,11-trimethylheptacosane was reported in fire ants (Nelson et al., 1980). These trimethylalkanes are present at trace levels in the G. (Glossina) taxa. In addition, internally branched monomethylalkanes are relatively less common in taxa of G. (Nemorhina) than in G. (Glossina).

The predominant internally branched di- and trimethylalkanes in adult males

and females are not correlated with putative subgeneric status. Adult males in both subgenera are characterized by 11,15- and/or 13,17-dimethylalkanes having 31- or 33-carbon backbones as the most abundant internally branched alkane(s). The predominant internally branched methylalkanes in adult females with *G. (Glossina)* or *G. (Nemorhina)* varies in the number and position of methyl branches present, the number of methylene units between methyl branches, and the backbone carbon number; however, closely related taxa have similar cuticular methylalkane compositions and patterns. It should be noted that complications are possible in that some compounds may be transferred between the sexes by contact.

Within *G. (Glossina)*, taxa can be divided into three groups delineated by the predominant internally branched alkanes in adult females. The dominant isomer in female *G. m. morsitans*, *G. m. centralis*, *G. m. submorsitans*, and *G. swynnertoni* is 15,19,23-trimethylheptatriacontane (Carlson et al., 1993), although the mass spectra for the last three taxa were not reported until this work. The presence of similar methylalkane compositions and relative abundances in these closely related taxa suggests that this compound may be involved in mediating sexual response in males of all members of this complex, although this has been shown experimentally only in *G. m. morsitans* (Huyton et al., 1980), as colonies of the related species are rare or unavailable. The major internally branched methylalkanes in *G. longipalpis* are the homologs 13,23-dimethylpentatriacontane and 13,23-dimethylheptatriacontane. The cuticular methylalkane composition of adult females of the morphologically and ecologically similar species, *G. pallidipes*, is dominated by 13,23-dimethylpentatriacontane, which has been implicated as a contact sex pheromone (Carlson et al., 1984). The remaining species within *G. (Glossina)*, *G. austeni*, does not fit into either of these two classes. The predominant internally branched alkanes in adult females of *G. austeni* were reported as mixtures of 11,*x*-, 13,*x*-, and 15,*x*-dimethylalkanes having three or five methylene units between branch points (Nelson and Carlson, 1986); however, the relative abundances of these compounds were low and sexual dimorphism poorly defined. In addition, there appears to be considerable intraspecific variation in the predominant isomer in females (Sutton, unpublished data). While males of *G. austeni* were reported to show a positive response to synthetic 15,19-dimethyltritriacontane (Huyton et al., 1980), repeated bioassays of the natural methylalkanes were inconclusive (P. A. Langley, personal communication). Recent bioassays of the alkene fraction of cuticular extracts (Carlson, unpublished data) have shown activity comparable to that of the sexually mature unmated female. This suggests that the cuticular mediators for male sexual response have shifted in *G. austeni* to the unsaturated analogs of the internally branched methylalkanes.

In *G. p. palpalis* females, 13,17,21-trimethylheptatriacontane is the most abundant isomer present, making up almost one third of the total methylalkane

composition (Nelson et al., 1988), and has been implicated in mediating sexual behavior (Offor et al., 1981), although bioassays of this compound have not shown full activity in comparison to total hydrocarbon extracts. It is possible that polar constituents of the cuticle in this species are also involved in promoting sexual response in males. The cuticular alkane composition of females of *G. f. martinii* is essentially identical with that reported for *G. f. fuscipes* (Nelson et al., 1988) in that 13,17,21?-trimethylheptatriacontane was predominant, although the 11,15,19?- isomer was also present at significant levels, where the position of the third methyl branch was ambiguous. It is possible that the 11,15,19- and/or 13,17,21-trimethylheptatriacontanes have similar bioactivity in all *G. palpalis* and *G. fuscipes*, perhaps in combination with yet to be determined polar compounds.

In *G. pallicera* females, in contrast, the relative proportion of the 11,15,19-, and 13,17,21-trimethylalkanes in the individuals examined was far lower, less than 5–15% of the total alkanes and the 11,15,19- isomer was predominant. In addition, the 11,15- and 13,17-dimethyl homologs approached the trimethylalkanes in abundance.

The two females of *G. caliginea* examined did not exhibit clearly defined sexual dimorphism in the amounts of internally branched alkanes present or a dominant isomer. The methylalkane isomers present were consistent with those seen in *G. pallicera* and *G. palpalis* (Nelson et al., 1988).

The remaining species, *G. tachinoides*, is unique within the *G. (Nemorhina)* in that the dominant methylalkanes in adult females are 37-carbon backbone dimethylalkanes of the form 11,*x*- and 13,*x*- with 9 and 11 methylene units between the branch points.

It is not clear whether the differences observed in the relative abundances of internally branched trimethylalkanes in adult females of *G. palpalis/G. fuscipes* and *G. pallicera/G. caliginea* represent sampling bias, such as age variation, or a fundamental shift in the pheromone system, as appears to have occurred in *G. austeni*. Marked sexual dimorphism in the predominant internally branched alkanes in *G. tachinoides*, 11,23- and 13,25-dimethylheptatriacontane, began three days before eclosion, but did not reach its maximum extent until three days or more after emergence (Messoussi et al., 1994). The small degree of differentiation in the relative abundances of di- and trimethylalkanes between male and female *G. pallicera* and *G. caliginea* in comparison to *G. palpalis* and *G. fuscipes* may reflect the degree of sexual maturation of the females available for analysis. Further work is needed to establish the presence or absence of a common pheromone system between these four species.

Within *G. (Glossina)* and *G. (Nemorhina)*, the interspecific variation in the internally branched alkanes in adult females implicated or suspected as mediating sexual response in males appears to represent a simple shift in isomer abundance rather than separate, de novo biosynthesis of each individual compound. In all

females, common patterns are observed that include several homologous series of internally branched di- and/or trimethylalkanes having backbone carbon numbers of 31–39. The predominant isomers are of the form 11,x-, 13,x-, 15,x-, 11,x,y-, 13,x,y-, and 15,x,y-, although it appears likely that complete di- and trimethylalkane series from 3,x- to 15,x- or 17,x- and 3,x,y-/4,x,y- to 15,x,y- or 17,x,y- exist in the cuticular lipids of all adults. In general, isomers having methyl branches close to an end position are most abundant at backbone carbon numbers below 31. The internally branched dimethylalkanes form two homologous series having 3 or 9 and/or 11 methylene units between the branch points. The former series is predominant in the males and has the mode at 31 or 33 carbons. The dimethylalkane series having 9 and/or 11 methylene units between branch points has the mode at 35 or 37 carbons and overlaps the former series in the region of 31–35 carbons. On high-resolution chromatographic columns such as the 50-m column used here with > 100,000 theoretical plates, these two series partially resolve at each carbon number, forming a bimodal peak. If dimethylalkane isomers having intermediate numbers of methylene units between branch points exist, i.e., $I = 5$ and/or 7, they are usually below detection threshold, except in *G. austeni*. The internally branched trimethylalkanes, especially those isomers involved in behavioral modification, are predominantly of an $I = 3,3$ pattern, although other isomers may be present. There is a general trend in that the internally branched di- and trimethylalkanes having biological activity in the tsetse flies are symmetrical (or nearly so) in terms of the methyl branch positions on the alkane backbone.

The differences in the major internally branched alkanes in adult females between species, or groups of species, in *G. (Glossina)* and *G. (Nemorhina)* represent shifts in the relative abundances of the two dimethylalkane series and the trimethylalkane series. In addition, quantitative shifts in isomer composition within a series and at a given backbone carbon number may occur and exhibit geographical trends as reported for *G. pallidipes* (Carlson et al., 1984). The aberrant *G. austeni* represents the greatest extreme in this pattern.

Sexual dimorphism in tsetse may also result from relative shifts in methylalkane abundance rather than separate, specialized biosynthesis of each internally branched di- and trimethylalkane in females. Quantification of the methylalkane composition in tsetse males is complicated by the potential transfer of cuticular components during mating from females. This phenomenon is known in laboratory colonies since unmated males possessed very small quantities of trimethylalkanes normally produced by females (Nelson and Carlson, 1986). Conversely, the transfer of species-specific cuticular alkenes from male to female during mounting was observed by GC in seven members of *G. (Glossina)* and *G. (Nemorhina)* (Carlson and Langley, 1986), and the absence of compounds produced by the opposite sex was noted in conspecific virgin flies. We utilized wild males that would be expected to contain no conspecific female-produced

compounds if naive but may have acquired materials during mating, although this appears unlikely because of low populations. Thus it is not possible a posteriori to determine whether the low levels of the long-chain internally branched alkanes found in situ are male-produced, since males that have never had contact with females after eclosion are not readily available except in the laboratory. In addition, it was suggested that unmated blood-fed female tsetse cannot be found in the field (P. A. Langley, personal communication), implying that males continue to attempt copulation with all females, regardless of their mated status, and thereby accumulate more than trace amounts of conspecific female-produced trimethylalkanes.

The methylalkanes described here have chiral potential, but are of unknown stereochemistry. Although this is a subject beyond the scope of this paper, chirality may contribute to the activity or inactivity of synthetic hydrocarbons made for biological studies. Resolution of natural enantiomers is likely to be difficult because of limited optical rotation, as well as their lack of chromophores or other "handles."

The cuticular alkane patterns and composition of the remaining taxa of the subgenus *Austenina* (the *fusca* group) will be reported in a subsequent paper.

APPENDIX 1: SPECIMENS EXAMINED

Glossina (Glossina)

G. morsitans centralis: males—West Tanzania, Tabora Region, Tabora District, Kubunde village (15 km NW Tabora town), alt. 1200 m, Igombe R., 8 April 1982 (N = 2) (FSCA); N. Rhodesia, Nawadia District, Kazemba's village, 21-IV-1904, W. P. Kennelly, 1905-1979. (NHM); Zambia, Central Province, E edge of Kafue fly belt on main road, 14°69'S 26°40'E, Davies, 16/7/82 (N = 2) (FSCA); females—West Tanzania, Tabora Region, Tabora District, Kubunde village (15 km NW Tabora town), alt. 1200 m, Igombe R., 8 April 1982 (N = 2) (FSCA); Egyptian Sudan, Mengalla District, 16-IX-1921, Capt. Neil Cantlie M.C., BM 1922-98 (NHM); Zambia, Central Province, E edge of Kafue fly belt on main road, 14°69'S 26°40'E, Davies, 16/7/82 (N = 2) (FSCA). *G. m. submorsitans:* males—Upper Volta (FSCA); Uganda, Gondokoro, 1913 (BM); Ranch de Madina, Reg: Sikasso, Mali, 22 September 1979, A. Cisse (N = 2) (FSCA); females—Upper Volta (FSCA); Nigeria (FSCA); Uganda, 2 miles north of Lake Nwamia, 1913 (NHM); Ranch de Madina, Reg:

Sikasso, Mali, 22 September 1979, A. Cisse (*N* = 2) (FSCA). *G. swynnertoni:* males—Tanganyika, Shinyanga, 1954 (*N* = 4) (FSCA); females—Tanganyika, Atasha, 13-VIII-1950, E. Burtt, BM 1959-638 (NHM); Tanzania, Lake Manyara, October 22, 1981, D. Carlson (FSCA); Tanganyika, Shinyanga, 1954 (*N* = 3) (FSCA): *G. longipalpis:* males—Ivory Coast, February 1987, T. Jordan (FSCA); N. Nigeria, R. Niger, Horin Province, nr. Jebba, October 1909, Dr. C. W. McLeay W.A.M.S. (NHM); Gold Coast, Volta River, March 1908, G. C. Dudgeon, 1908-201 (NHM); W. Ashanti (grass country), F. E. Fell, 13-VII-1911, 1911-220 (NHM); females—Ivory Coast, February 1987, T. Jordan (*N* = 3) (FSCA); Sebe, S. Nigeria, May 1908, G. C. Dudgeon, 1908-201 (NHM); Gwatehipi, Abuja, Niger Province, May 1961, Brit. Mus. 1962-678 (NHM); S. Nigeria, Lagos, G. C. Dudgeon, April 1908, 1908-201 (NHM)

Glossina (Nemorhina)

　　G. fuscipes martinii: males—L. Tanganyika, Mpulungu, 31-V-1948, E. Burtt, B.M. 1959-638 (NHM); Tanganyika, 1948, Dr. E. Burtt (NHM); Tanganyika, 1948, E. Burtt (unfed) (NHM); females—L. Tanganyika, Mpulungu, 12-VIII-1948, E. Burtt, B.M. 1959-638 (NHM); L. Tanganyika, 21.5.48 (NHM); Tanganyika, 1948, E. Burtt (unfed) (NHM): *G. pallicera pallicera:* males—Ashanti, Western Prov., on road between Lechiri and Lanuso, 25-XII-1911, M. Wade (NHM); Gold Coast, Ashanti Sunyani, 7.7.1914, A. Ingram (NHM); Ashanti, West Africa, October 27, 1907, W. M. Graham, 1908-245 (NHM); females—Ashanti, 1912, Watson (NHM); Ashanti, April 1912, J. E. Zell (NHM); Suji, Liberia, W. Africa, 21-V-1909, Major A. Pearse, R.A.M.C., 1909-184 (NHM): *G. p. newsteadi:* Males—Belgian Congo, Lower Somami R., R. Elipa, X-1929, J. Schwetz (NHM); NE Belgian Congo, R. Ubele, Monja Terr., May 1929, J. Schwetz (NHM); females—Belgian Congo, X-1929, J. Schwetz (NHM); NW Ashanti: Chirat to Sunyani, 9-XII-1911, J. E. Fell, Presented by Ent. Res. Committee (NHM): *G. caliginea:* Males—S. Nigeria, Ori R., 10-III-1911, W. A. Lamborn, 1911-462 (NHM) Douala, French Cameroons, J. Rageau, 11-IV-1950, Presented by Com. Inst. Ent. BM 1950-16 (NHM); Belgian Congo, 1959 (NHM); females—Missellele, nr. Tiko, B.C., November 1959, Brit. Mus. 1962-678 (NHM); French Cameroon, 1937 (NHM)

REFERENCES

CARLSON, D. A., and LANGLEY, P. A. 1986. Tsetse alkenes: Appearance of novel sex-specific compounds as an effect of mating. *J. Insect Physiol.* 32:781–790.

CARLSON, D. A., LANGLEY, P. A., and HUYTON, P. A. 1978. Sex pheromone of the tsetse fly: Isolation, identification and synthesis of contact aphrodisiacs. *Science* 201:750–753.

CARLSON, D. A., NELSON, D. R., LANGLEY, P. A., COATES, T. W., DAVIS, T. L., and LEEGWATER-

VANDER LINDEN, M. 1984. Contact sex pheromone in the tsetse fly *Glossina pallidipes* Austen: Identification and synthesis. *J. Chem. Ecol.* 10:429–450.

CARLSON, D. A., MILSTREY, S. K., and NARANG, S. K. 1993. Genetic classification of the tsetse flies using cuticular hydrocarbons. *Bull. Entomol. Soc.* 83:507–515.

HUYTON, P. M., LANGLEY, P. A., CARLSON, D. A., and SCHWARZ, M. 1980. Specificity of contact sex pheromones in tsetse flies, *Glossina* spp. *Physiol. Entomol.* 5:253–264.

KOVATS, E. 1965. Gas chromatographic characterization of organic substances in the retention index system. *Adv. Chromatogr.* 1:229–235.

LANGLEY, P. A., PIMLEY, R. W., and CARLSON, D. A. 1975. Sex recognition pheromone of the tsetse fly. *Nature* 254:51–53.

LANGLEY, P. A., COATES, T. W., and CARLSON, D. A. 1982. A sex recognition pheromone in the tsetse fly, *Glossina pallidipes* Austen. *Experientia* 38:473–475.

MARTIN, M. M., and MACCONNELL, J. G. 1970. The alkanes of the ant, *Atta colombica*, *Tetrahedron* 26:307–319.

MATSUYAMA, K., and MORI, K. 1994. Synthesis of a stereoisomeric mixture of 13,25-, 11,21- and 11,23-dimethylheptatriacontane, the contact sex pheromone of the tsetse fly, *Glossina tachinoides*. *Biosci. Biotech. Biochem.* 58:539–543.

MCDOWELL, P. G., WHITEHEAD, D. L., and CHAUDHURY, F. B. 1981. The isolation and identification of the cuticular sex-stimulant pheromone of the tsetse *Glossina pallidipes* Austen (Diptera: Glossinidae). *Insect Sci. Appl.* 2:181–187.

MESSOUSSI, S., WICKER, C., ARIENTI, M., CARLSON, D. A., and JALLON, J. M. 1994. Hydrocarbons in species recognition in insects, *in* D. L. Hawksworth (ed.). The Identification and Characterization of Pest Organisms. CAB International. Wallingford.

NELSON, D. R., and CARLSON, D. A. 1986. Cuticular hydrocarbons of the tsetse flies *Glossina morsitans*, *G. austeni* and *G. pallidipes*. *Insect Biochem.* 16:403–416.

NELSON, D. R., and SUKKESTAD, D. R. 1970. Normal and branched aliphatic hydrocarbons from eggs of the tobacco hornworm. *Biochemistry* 9:4601–4611.

NELSON, D. R., FATLAND, C. L., HOWARD, R. W., MCDANIEL, C. A., and BLOMQUIST, G. J. 1980. Re-analysis of the cuticular methylalkanes of *Solenopsis invicta* and *S. richteri*. *Insect Biochem.* 10:163–168.

NELSON, D. R., CARLSON, D. A., and FATLAND, C. L. 1988. Cuticular hydrocarbons of the tsetse flies, II: *G. p. palpalis*, *G. p. gambiensis*, *G. fuscipes*, *G. tachinoides*, and *G. brevipalpis*. *J. Chem. Ecol.* 14:963–987.

OFFOR, I. I., CARLSON, D. A., GADZAMA, N. N., and BOZIMO, H. T. 1981. Sex-recognition pheromone in the West African tsetse fly, *Glossina palpalis palpalis* (Robineau-Desvoidy). *Insect Sci. Appl.* 1:417–420.